

# Enthalpies of Solution of the Nucleic Acid Bases.

## 6. Guanine in Aqueous HCl and NaOH, and Guanine Hydrochlorides in Aqueous HCl

Marthada V. Kilday\*

National Bureau of Standards, Washington, D.C. 20234

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The following values of the enthalpy of solution of well-characterized samples of guanine were obtained from measurements in an adiabatic solution calorimeter:

Solvent	$\Delta H(\infty, 298.15 \text{ K})/\text{kJ}\cdot\text{mol}^{-1}$	$\Delta C_p/\text{J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$
HCl(aq) $\left\{ \begin{array}{l} \text{1st protonation} \\ \text{2nd protonation} \end{array} \right.$	30.181 $\pm$ 0.032	814 $\pm$ 75 (at $< 0.2 \text{ mol HCl}\cdot\text{L}^{-1}$ ) 98 $\pm$ 8 (0.3 to 3.5 mol HCl $\cdot\text{L}^{-1}$ ) 160 $\pm$ 15 (4.5 to 6.1 mol HCl $\cdot\text{L}^{-1}$ )
NaOH(aq)	13.545 $\pm$ 0.034	156 $\pm$ 8 (0.1 mol NaOH $\cdot\text{L}^{-1}$ )
H <sub>2</sub> O (calculated)	27.2 $\pm$ 2.1	

The following enthalpies of protonation were calculated for guanine:

	$\Delta H(\infty, 298.15 \text{ K})/\text{kJ}\cdot\text{mol}^{-1}$
Addition of 1 proton	3.0 $\pm$ 2.1
Addition of 2 protons	7.8 $\pm$ 2.2

Key words: Calorimetry;  $\Delta C_p$  of reaction; C<sub>5</sub>H<sub>5</sub>N<sub>5</sub>O; density; enthalpy of solution; Gua $\cdot$ HCl $\cdot$ 1 H<sub>2</sub>O; Gua $\cdot$ HCl $\cdot$ 2 H<sub>2</sub>O; Gua $\cdot$ 2 HCl; guanine (Gua); guanine hydrochlorides; heat capacity; nucleic acid base; solution calorimetry; thermochemistry.

### 1. Introduction

This is the final paper in this series [1-5].<sup>1</sup> It reports results from an incomplete study of the enthalpies of solution of guanine (Gua) and guanine hydrochlorides (Gua $\cdot$ nHCl) in aqueous solutions of hydrochloric acid and of sodium hydroxide. Although there are still assumptions and unanswered questions, the results that were obtained are being published because the calorimetry is of high accuracy and considerable information is given characterizing the samples used.

It was impossible to measure the enthalpy of solution of guanine in water directly because of its low solubility (0.04 g $\cdot$ L<sup>-1</sup> at 313 K [6]); however, a value at infinite dilution has

been calculated. Enthalpies of solution of guanine in HCl solutions of various concentrations from 0.01 to 6.1 mol $\cdot$ L<sup>-1</sup>, and in NaOH solutions from 0.09 to 3.1 mol $\cdot$ L<sup>-1</sup> were measured. Values for the  $\Delta C_p$  of reaction were also determined. Enthalpies of solution of anhydrous guanine hydrochlorides in aqueous HCl solutions (0.3 to 6.0 mol $\cdot$ L<sup>-1</sup>) were measured. Enthalpies at infinite dilution of protonation and of proton dissociation were calculated for guanine and compared with analogous values obtained for adenine [1,5], another purine base. The exact sites of the ionizations are somewhat uncertain.

### 2. The Samples

Calorimetric measurements are reported here for the enthalpies of solution of four guanine samples as received

\*Center for Thermodynamics and Molecular Science, National Measurement Laboratory

<sup>1</sup> Figures in brackets indicate literature references at the end of this paper.

from commercial sources<sup>2</sup> and one sample obtained from solution, precipitation, and recrystallization of one of the commercial products; and for one sample of guanine hydrochloride, and three samples derived from it by recrystallization. The following information about the samples from commercial sources was obtained from labels, catalogs, and brochures:

**Gua 1.** A 25-g, Grade A sample purchased about 1970 from Calbiochem, Cat. No. 3700, Lot 73219. Analysis: Nitrogen, 46.32 percent (dry basis); Moisture, 0.4 percent; Chromat. Homogeneous. Spectra at pH 2: 250/260, 1.37; 280/260, 0.84;  $\lambda_{max}$ , 273 m $\mu$ ;  $\epsilon_{max}$ , 7330 (dry basis);  $\lambda_{min}$ , 267 m $\mu$ ;  $\epsilon_{min}$ , 7200 (dry basis);  $\lambda_{max}$ , 248 m $\mu$ ;  $\epsilon_{max}$ , 11100 (dry basis).

**Gua 2.** Five 10-g samples obtained in November 1973 from Boehringer-Mannheim Corp. (made in Germany), Cat. No. 15516 NGAG, Kontrol-Nr. 7313507/ 1. Sept. 1975. Typical Analysis: Guanine (enzymatic) 98 percent, chromatographically pure. Stability: Store at room temperature; no detectable decomposition within 12 months.

**Gua 3.** A 5-g sample of guanine (2-aminohypoxanthine) for biochemistry from EM Labs. obtained in February 1974, Cat. No. 4221, Lot No. 2958531.

**Gua 4.** Four 25-g samples obtained in June 1974 from Calbiochem. Same as Gua 1 except Lot No. 400147.

**Gua·HCl 1.** A 25-g sample of the dihydrate obtained in February 1974 from Eastman Organic Chemicals, Cat. No. 1606, for chemical purposes, not for drug use. (This material had a pale yellow color.)

The following samples were derived from the commercial products by the methods described which are somewhat similar to those used by Stiehler and Huffman [7].

**Gua 4b.** Approximately 25 g of Gua 4 was dissolved in  $\sim 1$  L of a boiling solution containing 5 percent (by volume) of concentrated HCl. The resulting solution was cloudy and amber colored. To remove the color, several grams of activated coconut charcoal were added to the boiling solution. The hot solution was filtered in a polypropylene Buchner-type funnel with Whatman No. 50 above No. 1 filter paper and a layer of filter paper pulp on the top. The filtrate was clear, but still amber colored. To precipitate the free guanine, hot concentrated ammonium hydroxide was added dropwise to the boiling solution until pH 3 to 5 was reached using indicating paper. The precipitate was collected on Whatman No. 50 above No. 1 filter paper, and washed with hot water. The filtrate was clear and colorless. The product was again dissolved, reprecipitated, filtered, and washed with hot water until the washings gave no test for  $\text{Cl}^-$  with  $\text{AgNO}_3$  solution. This product was dried under vacuum over  $\text{Mg}(\text{ClO}_4)_2$  for about one week.

**Gua·HCl 2a, 2b, 2c, and 2d.** A 25-g portion of Gua 4 was dissolved, filtered, reprecipitated, filtered again, and washed with hot water as described above for Gua 4b. This product was again dissolved in the 5 percent ( $\sim 0.6 \text{ mol} \cdot \text{L}^{-1}$ ) HCl solution and filtered. The filtrate (0.6 L) containing the Gua·HCl was allowed to cool overnight. A large volume of needlelike crystals formed and were separated from the saturated liquid by

filtration. These crystals were dissolved slowly in a boiling solution containing 1.5 percent (by volume) of concentrated HCl. It was surprising that the crystals formed upon cooling this solution appeared to be of mixed composition—there were some yellowish plates or flakes mixed with small needles. The mixture was heated again; the boiling solution was cloudy and charcoal was added before filtering. The filtrate was clear and colorless. The free guanine was again precipitated, filtered, and washed. It was then dissolved in the hot 5 percent HCl solution. Again upon cooling, the abundance of needlelike crystals formed. About half (10 g) of these crystals were designated Gua·HCl 2a. The other half was redissolved in the 1.5 percent ( $\sim 0.2 \text{ mol} \cdot \text{L}^{-1}$ ) HCl solution, which, after cooling slowly, formed flaky crystals; this was designated Gua·HCl 2b. Both of these samples were dried for three days under vacuum in a desiccator containing  $\text{Mg}(\text{ClO}_4)_2$ . Gua·HCl 2a was resilient and did not break when tamped with a glass rod, but Gua·HCl 2b was brittle and the tamping produced a fine powder. Both of these samples were further dried for three days in a vacuum oven at  $\sim 340$  K. Half of the samples, 2a and 2b, were heated at 370 K in a vacuum oven for four days and designated 2c and 2d, respectively.

The characterization and analyses of these materials will be discussed in the following subsections.

## 2.1. Volatile Matter and Hygroscopicity

The volatile matter (presumably  $\text{H}_2\text{O}$ ) was determined from the loss in mass on heating in a vacuum oven where the samples were guarded by a liquid nitrogen trap from contamination by the oil vapors from the vacuum pump (the pressure was 1 kPa or less). The materials were heated at relatively low temperatures to reduce the possibility of decomposition. In table 1 the results of volatile matter determinations on samples of guanine and guanine hydrochlorides are given. Gua 1, 2, 3, 4, and Gua·HCl 1 are the commercial materials as received. Gua 4b was reprecipitated from Gua 4. Gua·HCl 2a and 2c were recrystallized from 5 percent ( $\sim 0.6 \text{ mol} \cdot \text{L}^{-1}$ ) HCl solution; Gua 2b and 2d were recrystallized from 1.5 percent ( $\sim 0.2 \text{ mol} \cdot \text{L}^{-1}$ ) HCl solution.

The samples heated at 340 K for 16 h or less, as listed in table 1, were spread out in flat aluminum dishes with tight fitting covers which were removed during heating. These samples were weighed after heating four h, then weighed again after each additional 2-h heating period. All other samples heated for longer periods were contained in glass weighing bottles or dishes.

Gua 2 differed from the other guanine samples in that there was a constant rate of loss in mass after the initial 4-h heating period. This was probably due to slow vaporization of some impurity. The other guanine samples were at constant mass after heating  $\sim 6$  h at 340 K.

One of the two samples of Gua 1 dried at 370 K (table 1) had been previously dried at 340 K and subsequently exposed to air under the controlled conditions in this laboratory (relative humidity,  $RH = 35 \pm 10$  percent, room temperature,  $T = 296 \pm 1$  K). The essentially equal loss in mass for the two samples on drying at 370 K suggests that the

<sup>2</sup> Commercial sources are named in this paper only for specific identification. The information presented is in no way intended as an endorsement or a condemnation by the National Bureau of Standards of any of the materials or services.

Table 1. Determination of volatile matter (or H<sub>2</sub>O) in various samples of guanine and guanine hydrochlorides

Sample		Heating		<sup>a</sup> Mass Lost
Designation	Mass	Time	Temperature	
	g	h	K	%
Gua 1	2.20518	12	340	0.77 (0.75)
	3.10806	12	340	.77 (0.75)
	4.02280	12	340	.78 (0.75)
	1.53844	48	370	.96
	2.22628	48	370	<sup>b</sup> 1.01
Gua 2	1.98750	12	340	<sup>c</sup> 1.45 (1.41)
	2.01022	12	340	<sup>c</sup> 1.47 (1.42)
	2.06132	12	340	<sup>c</sup> 1.46 (1.42)
Gua 3	1.01588	12	340	1.52 (1.41)
	1.09410	12	340	1.48 (1.36)
	1.04799	12	340	1.49 (1.38)
Gua 4	3.78185	48	370	1.70
Gua 4b	3.33798	48	370	0.84
<sup>d</sup> Gua·HCl 1	2.24860	10	340	<sup>e</sup> 8.80 (8.4)
	2.64132	16	340	<sup>e</sup> 8.85 (8.4)
	3.03390	12	340	<sup>e</sup> 8.84 (8.4)
	17.550	70	340	14.2
Gua·HCl 2a	6.590	70	340	0.76
Gua·HCl 2b	5.895	70	340	0.59
<sup>f</sup> Gua·HCl 2c	3.205	95	370	< 0.03
<sup>g</sup> Gua·HCl 2d	2.240	95	370	0.36

<sup>a</sup> Values given are for the total heating time, those in parentheses are for the mass lost after heating 4 h.

<sup>b</sup> This sample was previously dried at 340 K and subsequently exposed to air (T = 296 K, RH = 35 %).

<sup>c</sup> After the initial 4-h heating period, this material lost mass at the constant rate of 0.1 mg·h<sup>-1</sup> for an additional 8 h. The other guanine samples were at constant mass after heating a total of 6 h.

<sup>d</sup> This material was nominally the dihydrate which was confirmed by density measurements. Theoretical H<sub>2</sub>O = 16.1 %.

<sup>e</sup> An additional 0.5 % loss occurred after heating 24 h at 370 K (total loss = 9.3 %; removal of 1 H<sub>2</sub>O of hydration corresponds to a loss of 8.0 %).

<sup>f</sup> This was a portion of Gua·HCl 2a which had been dried at 340 K.

<sup>g</sup> This was a portion of Gua·HCl 2b which had been dried at 340 K.

moisture lost on drying at 340 K was reabsorbed upon exposure to air. Therefore, the calorimetric samples of guanine were transferred in the room air and corrected for the moisture found as volatile matter.

Two H<sub>2</sub>O determinations on Gua 1 and 2 by Karl Fischer titrations (the method was previously described [1]) indicated 0.64 and 1.05 percent H<sub>2</sub>O, respectively. These values are not considered to be as reliable as the volatile matter determinations for small amounts of H<sub>2</sub>O, especially because the guanine was only slightly soluble in the methanol solvent.

The guanine hydrochloride, Gua·HCl 1, as received from the commercial source, was nominally the dihydrate. It was hygroscopic; a gain in mass of 0.1 percent was observed in a period of 5 min exposure to room air (RH = ~ 35%). All

subsequent operations transferring this material were performed in dry atmospheres. The first 3 samples of Gua·HCl 1 listed in table 1 were in Al dishes and lost a total of 9.3 percent H<sub>2</sub>O (8.4% in the first 4 h at 340 K) which is greater than the theoretical loss for one hydration H<sub>2</sub>O (8.0%). Another larger sample in a glass dish heated at 370 K for ~ 70 h lost mass nearly equivalent to 2 H<sub>2</sub>O. Thus it appears that the second water of hydration is removed relatively slowly upon heating at this temperature.

Gua·HCl 2a and 2b were products of recrystallization of reprecipitated Gua 4 from HCl solutions (0.6 mol·L<sup>-1</sup> and 0.2 mol·L<sup>-1</sup>, respectively). Both products were dried overnight at 296 K under vacuum in a desiccator containing Mg(ClO<sub>4</sub>)<sub>2</sub>. It appears that this removed most of the water of hydration, because vacuum drying at 340 K for 70 h resulted in a mass loss of less than 1 percent (see table 1). About half of these dried products were further dried 95 h at 370 K and are designated Gua·HCl 2c and 2d, respectively, in table 1.

Small portions of the guanine hydrochloride samples listed in table 1 as dried for 70 h or longer were transferred (in a glove box containing a desiccant) to weighing bottles for hygroscopicity observations. After an initial weighing, the caps to the bottles were removed and the samples were exposed to the room air while standing in the balance case. The weights were recorded at various intervals over a period of three days. The cumulative changes in mass for the 5 samples are shown in table 2. Gua·HCl 2a and 2c were not hygroscopic under these conditions, but Gua·HCl 2b and 2d absorbed moisture for several hours. The moisture absorbed was approximately equal to that lost on heating and

Table 2. Cumulative mass changes observed upon exposure of dried Gua·HCl samples to air (RH = ~ 35 % and 296 K).

Δt	Gua·HCl sample				
	1	2a	2b	2c	2d
min			mg		
15	1.7	0.0	0.5	-0.1	0.4
85	7.5	.0	1.8	.0	1.5
205	6.3	.0	2.2	.0	1.8
1310	31.5	- .1	2.4	.0	2.0
2785	32.8	- .3	2.5	.0	2.1
4405 (~ 3 days)	33.4	- .6	2.4	- .2	2.0
Sample Mass, mg	559	340	284	211	221
Total Δ mass, %	6.0	-0.2	0.8	0.1	0.9

is apparently not water of hydration. Gua·HCl 1 (originally the dihydrate) was still absorbing moisture after 3 days and the 6 percent gain in mass was approaching the 8 percent equivalent of 1 H<sub>2</sub>O.

All calorimetric samples of the guanine hydrochlorides were the dried material. Transfers to the sample holder were made in a dry atmosphere to prevent absorption of moisture by the samples.

## 2.2. Elemental Analysis of the Guanine Hydrochlorides

Portions of the guanine hydrochloride samples which had been heated under vacuum for 70 h or longer (see table 1) were transferred in a glovebox to glass vials with tight-fitting plastic caps for shipment to the analytical laboratory. Four days later, the microanalyses were performed; the results are given in table 3. The compositions of Gua·HCl 1 and 2c correspond to that of the anhydrous monohydrochloride, but that of Gua·HCl 2d is closer to the hemihydrochloride. This might be expected since the latter sample was recrystallized from a more dilute HCl solution than Gua·HCl 2c.

Although the compositions of Gua·HCl 1 and 2c (table 3) are similar, they must be of different structure because it was shown in section 2.1 that the Gua·HCl 1 was very hygroscopic and the Gua·HCl 2c was not. It is important that the dried guanine hydrochlorides are anhydrous. Only

the dried materials were used in the calorimetric experiments. It will be assumed that samples 1 and 2c are the anhydrous monohydrochloride and that sample 2d is the anhydrous hemihydrochloride although these may be erroneous assumptions.

## 2.3. Density

The densities of several samples of guanine and the guanine hydrochlorides were measured by a displacement method in 25-cm<sup>3</sup>, Gay-Lussac-type pycnometers using Eastman ACS spectroscopic grade CCl<sub>4</sub> (density = 1.5898 g·cm<sup>-3</sup> under the laboratory conditions; this is the mean of 3 measurements for which the average deviation was 0.0002 g·cm<sup>-3</sup>). Details of the method are described in the first paper of this series [1]. The results of these measurements are given in table 4.

After the density measurement for Gua 1, the sample was collected on filter paper, dried in air, and weighed; there was a loss in mass of ~ 1 mg or < 0.1 percent. When the filtrate was evaporated to dryness there was no visible residue. Thus, the solubility of guanine in CCl<sub>4</sub> was insignificant in the density determinations.

The results of density measurements given in table 4 for Gua 1, 3, 4, and Gua·HCl 1 were obtained on samples as received from commercial sources; the other measurements were made on the dried samples (see sec. 2.1). For calculating the buoyancy factor, 1.000546, used in this work to cor-

Table 3. Microanalysis<sup>a</sup> of 3 dried samples of the guanine hydrochlorides and theoretical compositions of some probable components.

Sample Designation	Empirical Formula	<sup>b</sup> Molar Mass	C	H	O	N (Kjeldahl)	Cl
		g/mol	per cent				
Gua·HCl 1			31.73	3.47	8.88	37.26	18.34
Gua·HCl 2c			31.82	3.04	8.60	36.61	18.72
Gua·HCl 2d			36.07	3.56	10.33	41.85	7.66
Theoretical Composition:							
Gua	C <sub>5</sub> H <sub>5</sub> N <sub>5</sub> O	151.1274	39.74	3.33	10.59	46.34	0.00
Gua·1/2 HCl	C <sub>5</sub> H <sub>5.5</sub> N <sub>5</sub> OCl <sub>0.5</sub>	169.3578	35.46	3.27	9.45	41.35	10.47
Gua·1 HCl	C <sub>5</sub> H <sub>6</sub> N <sub>5</sub> OCl	187.5883	32.01	3.22	8.53	37.33	18.90
Gua·2 HCl	C <sub>5</sub> H <sub>7</sub> N <sub>5</sub> OCl <sub>2</sub>	224.0492	26.80	3.15	7.14	31.26	31.65
Gua·HCl·1 H <sub>2</sub> O	C <sub>5</sub> H <sub>8</sub> N <sub>5</sub> O <sub>2</sub> Cl	205.6035	29.21	3.15	15.56	34.06	17.24
Gua·HCl·2 H <sub>2</sub> O	C <sub>5</sub> H <sub>10</sub> N <sub>5</sub> O <sub>3</sub> Cl	223.6187	26.86	4.51	21.46	31.32	15.85

<sup>a</sup> Analyses by Micro-Analysis, Inc., Wilmington, DE

<sup>b</sup> Obtained from 1975 Table of Atomic Weights [12].

Table 4. Densities of Gua and Gua·HCl samples measured by a displacement method.

Sample Designation	Mass	Density
	g	g/cm <sup>3</sup>
Gua 1	1.3	1.725
Gua 3	1.3	a,b 1.727
Gua 4	0.8	1.702
Gua 4b	1.3	1.720
Gua·HCl 1	0.5 0.4	a 1.56 1.54
Gua·HCl 2a	0.3	1.64
Gua·HCl 2c	0.3 0.4	1.64 1.64
Gua·HCl 2d	0.2 0.3	c 1.53 c 1.50

<sup>a</sup> A few floating particles were observed.

<sup>b</sup> Sample appeared wet by CCl<sub>4</sub> with little degassing.

<sup>c</sup> Vigorous bubbling apparently from degassing.

rect the guanine weights to vacuum, the density of guanine, 1.72 g·cm<sup>-3</sup> was taken from the results in table 4.

This method for determining densities proved unsatisfactory for the guanine hydrochlorides. The densities measured did not agree with the elemental analyses. It was suspected that impurities in the CCl<sub>4</sub> reacted with the dried samples to change their compositions during the density measurements.

It was reported in Beilsteins Handbuch[8] that the hydrogen chloride salt of guanine may crystallize as the monohydrate (molar mass = 205.60), as the dihydrate (molar mass = 223.62), or as guanine dihydrochloride (molar mass = 224.05). Broomhead [9] prepared two crystalline guanine hydrochloride samples. The first was found to have molar mass = 223, density = 1.562 g·cm<sup>-3</sup>, and microanalysis indicated that it was the monohydrochloride dihydrate rather than the dihydrochloride. The second sample had density = 1.662 g·cm<sup>-3</sup> and molar mass = 205.2, and was apparently the monohydrate. (Details of the preparations and measurements were not given.)

Our density measurements confirmed that Gua·HCl 1 (as received) was the dihydrate (although the anhydrous material prepared from it was used in the calorimetric experiments). However, the measurements on the dried samples did not agree with the elemental analyses. Therefore, these

density values were used only for calculating buoyancy factors for determining the mass of samples; the resulting errors are relatively small.

## 2.4. Other Analyses

The heat capacities at 298 K,  $C_p$ , of two crystalline guanine samples were measured by Ernesto Friere using a drop microcalorimeter at the University of Virginia. He obtained 1.063 J·g<sup>-1</sup>·K<sup>-1</sup> for Gua 1 and 1.079 J·g<sup>-1</sup>·K<sup>-1</sup> for Gua 4b with an estimated uncertainty of 0.008 J·g<sup>-1</sup>·K<sup>-1</sup> for the measurements [10].

Emission spectrochemical analyses<sup>3</sup> of samples of Gua 1, 2, 3, and 4 revealed no impurities in the samples greater than the limits of detection reported for adenine [1].

Analysis of gas chromatograms<sup>4</sup> obtained from trimethylsilylation of Gua 1, 2, 3, 4, and 4b led to the conclusion that Gua 1 and 3 appear to have somewhat less impurity than the others. No specific conclusions could be drawn from these measurements.

The following information was reported from analysis<sup>5</sup> of X-ray powder diffraction patterns. Gua 1, Gua 3, Gua 4, and Gua 4b all produced weak patterns which could be identified as crystalline guanine. No major impurities were found. Gua 3 produced the pattern with greatest detail. The three samples of guanine hydrochlorides, Gua·HCl 1, 2c, and 2d, were all crystalline. The pattern from Gua·HCl 1 was distinctly different from those of 2c and 2d, which were sufficiently similar to indicate that they had the same crystallographic lattice. It is not possible to draw conclusions about the states of hydration.

No impurities were identified or detected from analyses by paper and thin layer chromatography (TLC) in the samples of Gua 1, 2, 3, 4, and 4b. Details of the procedures and detection limits were described previously [1]. The spotting solutions contained ~ 0.01 mol Gua/L of aqueous HCl or NaOH (1 mol·L<sup>-1</sup>). (Guanine was nearly insoluble in the hot NH<sub>4</sub>OH used for the other bases). The  $R_f$  values (distance traveled by the major component/distance traveled by the solution) were determined for the 5 guanine samples using TLC plates coated with 250 nm of MN300 Cellulose(C), or (CF) which also contains a fluorescent indicator, and Whatman No. 1 and No. 40 chromatography papers, and 4 different carrier solutions described in table 5. The  $R_f$  values obtained for guanine samples are compared with those given by the National Academy of Sciences (NAS) [11] for similar solutions and procedures. The NAS values are lower than in

<sup>3</sup> Analyses by J. A. Norris, Gas and Particulate Science Division, Center for Analytical Chemistry, National Measurement Laboratory.

<sup>4</sup> Analysis by D. Enagonio, Organic Analytical Research Division, Center for Analytical Chemistry, National Measurement Laboratory.

<sup>5</sup> Analysis by Camden R. Hubbard, Crystallography Section, Ceramic, Glass, and Solid State Science Division, National Measurement Laboratory.

Table 5.  $R_f$  values for 5 guanine samples using TLC plates and papers with 4 carrier solutions, A,B,C, and D<sup>a</sup>.

Gua Sample	Solvent	A			B				C			D			
		b <sub>C</sub>	CF	P-40	C	CF	P-1	P-40	C	CF	P-40	C	CF	P-1	P-40
1	HCl(aq, 1 mol·L <sup>-1</sup> )	0.64	0.77	0.73	0.47	0.16	0.40	0.50	0.46	0.25	0.54	0.53	0.32	0.45	0.42
2		.66	.76	.76	.51	.16	.43	.50	.47	.25	.52	.57	.34	.43	.44
3		.67	.76	.76	.52	.16	.41	.48	.46	.25	.54	.57	.33	.44	.45
4		.69	.76	.74	.51	.18	.41	.48	.48	.25	.54	.56	.32	.45	.47
4b		.66	.75	.72	.51	.13	.43	.48	.46	.26	.56	.54	.32	.45	.47
NAS [11]				.66				.27			.47				.40
1	NaOH(aq, 1 mol·L <sup>-1</sup> )	---	0.76	0.71	---	0.25	0.36	0.58	---	0.19	0.47	---	0.86	0.50	0.49
2		---	.73	.72	---	.26	.39	c	---	.18	.38	---	.83	.49	.50
3		---	.71	.73	---	.26	.34	c	---	.19	.36	---	.86	.47	.51
4		---	.74	.72	---	.25	.35	c	---	.18	.42	---	.85	.48	.50
4b		---	.73	---	---	.26	.36	c	---	.17	.41	---	.84	.48	.50

<sup>a</sup> The volume compositions of the carrier (or tank) solutions was as follows:

Soln A: 5 parts of iso-butyric acid + 3 parts of NH<sub>4</sub>OH(aq, 0.5 mol·L<sup>-1</sup>).

Soln B: 7 parts of iso-propyl alcohol + 1 part of conc. NH<sub>4</sub>OH + 2 parts of H<sub>2</sub>O.

Soln C: 7 parts of 95 % ethyl alcohol + 3 parts of sodium acetate (aq, 1 mol·L<sup>-1</sup>).

Soln D: H<sub>2</sub>O adjusted to pH 10 with NH<sub>4</sub>OH (~ 1 drop of conc. NH<sub>4</sub>OH in 300 mL H<sub>2</sub>O).

<sup>b</sup> C is a glass TLC plate coated with 250 μm of MN300 cellulose.

CF is the same as C except it includes a fluorescent indicator.

P-1 and P-40 are Whatman No. 1 and No. 40 chromatography papers.

<sup>c</sup> Bands at  $R_f$  = ~ 0.48 instead of spots.

this work, but are still within the uncertainties to be expected under differing experimental conditions. This was also true for the  $R_f$  values reported previously in this series [1-4].

### 3. Enthalpy of Solution Measurements

The relative molar masses used in this work were obtained from the 1975 Table of Atomic Weights [12]; the unit of energy is the joule as defined in the International System of Units (SI). Uncertainties are expressed as standard deviations except as otherwise noted. The weights in air for all crystalline samples and solutions have been corrected to vacuum (mass) as described previously [13]. For calculating buoyancy factors, densities for the aqueous HCl and NaOH solutions were obtained from the Handbook of Chemistry and Physics [14].

Enthalpy of solution measurements were made in a platinum-lined silver, adiabatic, vacuum-jacketed, solution calorimeter. The calorimeter, procedures used for measurements, electrical measuring instruments, and corrections to the experimental data have been described [13, 15].

The calorimetric measurements reported here were made intermittently between November 1973 and April 1975. The stirring rate was 700 revolutions per minute (rpm) for all ex-

periments using Gua and Gua·HCl in HCl solutions (except Nos. 888, 889, and 891 at 450 rpm). The rate was 450 rpm for the measurements of Gua in NaOH solutions.

The following definitions apply to expressions in the text and in the tables:

Expt. No. is a serial number for experiments with this calorimeter and indicates the chronological order of the experiments.

Reaction period is the elapsed time between the initiation of the reaction and the beginning of the rating period which follows the reaction. Occasionally, there are long reaction periods which are probably the result of the way the sample holder opens. If the cylinder containing the sample does not tilt slightly, some of the sample may remain in it and dissolve very slowly. However, since heat leak corrections have been shown to be negligible in this calorimeter, the rate of the reaction does not seriously affect the results.

$\epsilon_i$  and  $\epsilon_f$  are the electrical energy equivalents of the initial and final systems.

$\Delta T_{corr}$  is the observed temperature change corrected for constant energy sources such as stirring energy.

$Q_{obs}$  is the observed heat of reaction;

$$Q_{obs} = \left( \frac{\epsilon_i + \epsilon_f}{2} \right) \Delta T_{corr.}$$

$Q_v$  is the correction for vaporization of water into the air space contained in the sample holder;

$$-q_v = \Delta H_v \left( V - \frac{s}{d} \right) (1 - RH)$$

where  $\Delta H_v$  is the enthalpy of vaporization of water/unit volume at the mean temperature of the reaction [16],  $V$  is the internal volume of the sample holder (in this work, 0.74 cm<sup>3</sup>),  $s$  is the mass of sample,  $d$  is the density of the sample, and  $RH$  is the relative humidity of the atmosphere in which the sample was transferred to the sample holder ( $RH = \sim 0.35$  for the room air or  $RH = \sim 0$  in the glove box).

$q_{diln}$  is a correction for dilution by the sample water of the HCl solutions. This correction was negligible ( $n$ ) in the dilute HCl solutions ( $< 1 \text{ mol} \cdot \text{L}^{-1}$ ) and in the NaOH solutions. Enthalpies of dilution for the more concentrated HCl solutions were taken from Parker's tabulation [17].

$Q_r$  is the specific corrected heat of the reaction;

$$Q_r = (Q_{obs} - q_v - q_{diln}) s^{-1}.$$

$T_r$  is the mean temperature of the reaction.

$\text{Corr}_{ST}$  is the correction to the standard or reference temperature, 298.15 K;

$$\text{Corr}_{ST} = \Delta c_p (T_r - 298.15 \text{ K})$$

where the values for  $\Delta c_p$ , the change in specific heat capacity during the reaction, are determined in this work as

$$\Delta c_p = - \left( \frac{Q_{rT_1} - Q_{rT_2}}{T_2 - T_1} \right).$$

The values of  $Q_r$  are from 2 experiments at different temperatures,  $T_1$  and  $T_2$ . The specific enthalpy of solution at the reference temperature is  $-(Q_r - \text{Corr}_{ST})$ ; the molar enthalpy of solution is

$$\Delta H(298.15 \text{ K}) = -(Q_r - \text{Corr}_{ST})M$$

where  $M$  is the relative molar mass of the sample.

### 3.1. $\Delta H_{soln}$ of Gua in Aqueous HCl

Gua 1 was used for most of the measurements of the enthalpy of solution because it was white crystalline material which appeared to be relatively free of impurities and the

volatile matter found was less than in the other three samples from commercial sources. It was assumed that the discoloration in Gua 3 was from impurity although none was detected or identified in the analytical work described in section 2. Gua 4 was also slightly discolored and was used in preparing other samples of guanine and guanine hydrochlorides by reprecipitation and recrystallization. A few measurements of the enthalpy of solution were made with samples other than Gua 1 for comparison.

In table 6 are the data from the measurements of the enthalpy of solution in HCl solutions of various concentrations; the arrangement of the experiments is in order of increasing HCl concentration. The mass of guanine was  $\sim 0.2 \text{ g}$  (1.2 to 1.5 mmol) and the volume of the solution was  $\sim 300 \text{ cm}^3$  in all experiments. The experiments are divided into 3 groups because the measured values for the  $\Delta c_p$  of the reaction were significantly different at different HCl concentrations.

Table 7 shows the Expt. Nos. (from table 6) for which values of  $Q_r$  and  $T_r$  were used in calculating  $\Delta c_p$  for the reaction at five concentrations of HCl. The  $\Delta c_p$  for group 1,  $5.39 \text{ J} \cdot \text{g}^{-1} \cdot \text{K}^{-1}$ , was derived from only one pair of experiments at the concentration,  $0.1013 \text{ mol} \cdot \text{L}^{-1}$ . It was applied in the calculation of  $\text{Corr}_{ST}$  for this entire group because a precipitate formed in the final solutions of all these experiments, but not in those of Groups 2 and 3. At first, this undissolved material was believed to be unreacted guanine. However, observation of a non-calorimetric experiment revealed the immediate formation of a voluminous white precipitate (presumably the hydrochlorides since there was an excess of  $\text{Cl}^-$  in even the most dilute solution), only part of which dissolved in the dilute solutions. The concentration where the dissolution of this precipitate was complete is shown graphically in figure 1 which is a plot of the values given in table 6 for  $\Delta H(298.15 \text{ K})$  as a function of HCl concentration. The values for the enthalpy of solution increase sharply with increasing HCl concentration until a precipitate is no longer visible in the final solution,  $\sim 0.2 \text{ mol} \cdot \text{L}^{-1}$ .

The values measured for  $\Delta c_p$  of the reaction (table 7) at the HCl concentrations, 0.307 and  $3.46 \text{ mol} \cdot \text{L}^{-1}$ , agreed, and the mean value,  $0.65 \text{ J} \cdot \text{g}^{-1} \cdot \text{K}^{-1}$ , was used in calculating  $\text{Corr}_{ST}$  for the experiments in Group 2. At HCl concentrations, 5.45 and  $6.09 \text{ mol} \cdot \text{L}^{-1}$ , the values for  $\Delta c_p$  also agreed, and the mean value,  $1.06 \text{ J} \cdot \text{g}^{-1} \cdot \text{K}^{-1}$ , was used to obtain  $\text{Corr}_{ST}$  for experiments in Group 3. It was questionable whether the  $\Delta c_p$  value from Group 2 or 3 should be used for the 6 experiments using HCl solutions between the concentrations, 3.46 and  $5.45 \text{ mol} \cdot \text{L}^{-1}$ . The use of either value for  $\Delta c_p$  resulted in lower values for  $\Delta H(298.15 \text{ K})$  than would correspond to the curve for Group 2 (see fig. 1). Therefore, it was assumed that the second protonation occurred at the HCl concentration of  $4 \text{ mol} \cdot \text{L}^{-1}$  or less, and the 6 experiments in question were included in Group 3.

Table 6. Data from the measurements of the enthalpy of solution of guanine in aqueous HCl solutions.

Expt No.	No.	Gua Mass <sup>a</sup> g	No. b	HCl Conc. mol/L	Soln. Mass g	Reaction Period min	Electrical Energy Equivalent		-ΔT <sub>corr</sub> K	-Q <sub>obs</sub> J	-q <sub>v</sub> J	-q <sub>diln</sub> J	-Q <sub>r</sub> <sup>c</sup> J/g	T <sub>r</sub> k	Corr <sub>ST</sub> J/g	ΔH(298.15 K)	
							ε <sub>i</sub>	ε <sub>f</sub>								J/g	kJ/mol
Group 1:																	
982	1	0.20262	(54)	0.0101	302.82	12	1737.20	1736.98	0.001726	2.998	0.02	n	14.20	298.195	0.24	14.46	2.18
981	1	.19803	(54)	.0251	303.17	17	1737.57	1737.36	.004146	7.204	.02	n	36.28	298.188	0.20	36.08	5.45
980	1	.21185	(54)	.0507	302.50	67	1733.28	1732.83	.008982	15.566	.02	n	73.38	298.173	0.12	73.26	11.07
978	1	.22297	54	.1013	302.47	47	1728.67	1728.47	.015982	27.626	.02	n	123.81	298.173	0.12	123.69	18.69
979	1	.21837	54	.1013	302.48	52	1728.18	1727.93	.023053	39.837	.04	n	182.24	309.013	(used for ΔC <sub>p</sub> value)		
983	1	.21740	50	.183	302.42	57	1721.85	1721.44	.023552	40.548	.02	n	186.42	298.173	0.12	186.30	28.16
Group 2:																	
977	1	0.21403	51	0.307	302.47	27	1712.42	1711.75	0.024258	41.532	0.02	n	193.95	298.139	-0.01	193.96	29.31
1028	1	.19891	51	.307	302.49	12	1713.17	1712.41	.023388	40.059	.04	n	201.19	308.996	7.05	194.14	29.34
1042	2	.20598	52	.312	307.44	32	1731.80	1731.19	.022700	39.305	.02	n	190.72	297.878	-0.18	190.90	28.85
1044	3	.20677	52	.312	307.46	122	1731.51	1730.99	.021935	37.975	.02	n	183.56	297.880	-0.18	183.74	27.77
984	1	.20260	55	.787	302.46	7	1676.00	1676.00	.022348	37.455	.02	n	184.85	298.233	0.05	184.80	27.93
1025	4b	.20188	55	.787	302.48	22	1676.09	1674.73	.021279	35.651	.02	---	176.50	298.118	-0.02	176.52	26.67
1026	4	.19267	55	.787	302.45	12	1675.32	1674.70	.020364	34.110	.02	n	176.93	298.222	0.05	176.88	26.73
976	1	.20089	56	.997	307.43	12	1680.61	1680.54	.021528	36.179	.02	n	180.13	298.106	-0.03	180.17	27.23
1024	4b	.19758	56	.997	307.47	22	1679.14	1678.53	.020356	34.174	.02	---	172.86	297.916	-0.15	173.01	26.15
1027	4	.19492	56	.997	307.48	27	1680.13	1679.16	.019276	32.380	.02	n	166.01	298.152	0.00	166.01	25.09
1036	1	.20387	(59)	3.46	307.95	37	1537.65	1537.48	.017645	37.130	.07	0.03	132.58	319.212	13.7	118.9	17.97
1037	1	.19450	(59)	3.46	308.00	97	1531.33	1530.54	.016095	24.640	.04	.03	126.33	309.242	7.20	119.12	18.00
Group 3:																	
1038	1	0.19831	(60)	4.49	307.87	32	1484.56	1484.76	0.013859	20.576	0.07	0.05	103.15	319.198	22.3	80.8	12.22
1039	1	.19818	60	5.01	307.44	17	1457.88	1458.00	.012060	17.583	.07	.09	87.91	319.201	22.3	65.6	9.92
1032	1	.19996	59	5.06	302.45	142	1421.17	1421.01	.009143	13.377	.02	.09	66.35	298.002	-0.16	66.51	10.05
1021	1	.19638	58	5.13	302.42	131	1417.64	1418.16	.008475	12.017	.02	.09	60.63	298.251	0.11	60.52	9.15
1022	4	.20424	58	5.13	302.46	22	1418.07	1418.40	.007594	10.770	.02	.09	52.19	298.119	-0.03	52.22	7.89
1023	4b	.20133	58	5.13	302.44	42	1417.09	1417.78	.008161	11.568	.02	---	57.36	297.443	-0.08	57.44	8.68
1040	1	.19648	(61)	5.45	313.46	112	1438.37	1438.54	.007112	10.230	.04	.11	51.40	298.109	-0.05	51.35	7.76
1041	1	.19875	(61)	5.45	313.42	22	1449.04	1448.76	.008864	12.843	.04	.11	63.86	309.116	11.6	52.24	7.90
1043	2	.19478	61	5.98	312.35	27	1422.42	1422.03	.005620	7.993	.04	.22	39.70	309.002	11.5	28.20	4.26
888	1	.18419	47	6.09	312.37	32	1407.85	1407.94	.004037	5.684	.02	.16	29.88	298.188	0.04	29.84	4.51
889	1	.19862	47	6.09	312.33	22	1407.51	1407.67	.004250	5.982	.02	.18	29.11	298.244	0.10	29.01	4.38
891	1	.19344	47	6.09	312.33	12	1417.77	1418.55	.005625	7.977	.04	.17	40.15	308.889	11.4	28.77	4.35

<sup>a</sup> Corrections to the mass of guanine for volatile matter (H<sub>2</sub>O) reported in sec. 2.1 are as follows: Gua 1, 1.0 %, Gua 2 and 3, 1.5 %; and Gua 4, 1.7 %. No corrections were made to Gua 4b because the dried sample was used in the measurements.

<sup>b</sup> HCl Soln. No. is the number of the stock solution from which the calorimetric solution was taken. The numbers in parentheses indicate that the stock solution was diluted to obtain the calorimetric solution.

<sup>c</sup>  $-Q_r$  includes corrections of - 0.027 J (No. 976) and - 0.014 J (No. 984) for brief departures of the shield temperature from that of the vessel because the reaction was fast.

<sup>d</sup> The measured values for  $\Delta C_p$  at different HCl concentrations did not agree, therefore the following values were used for corrections to the reference temperature: 5.4 J·g<sup>-1</sup>·K<sup>-1</sup> for Group 1; 0.65 J·g<sup>-1</sup>·K<sup>-1</sup> for Group 2; and 1.06 J·g<sup>-1</sup>·K<sup>-1</sup> for Group 3.



Table 7. Values of  $\Delta c_p$  for the reaction of Gua 1 in HCl solutions of various concentrations. (The uncertainties are estimated.)

Expt. Nos.	HCL conc.	$\Delta c_p$	Mean	Group
	mol/L	J/g·K	J/g·K	
978 and 979	0.1013	$5.39 \pm 0.50$	---	1
977 and 1028	0.307	0.67	$0.65 \pm 0.05$	2
1036 and 1037	3.46	0.63		
1040 and 1041	5.45	1.13	$1.06 \pm 0.10$	3
891 and mean of 888 and 889	6.09	1.00		

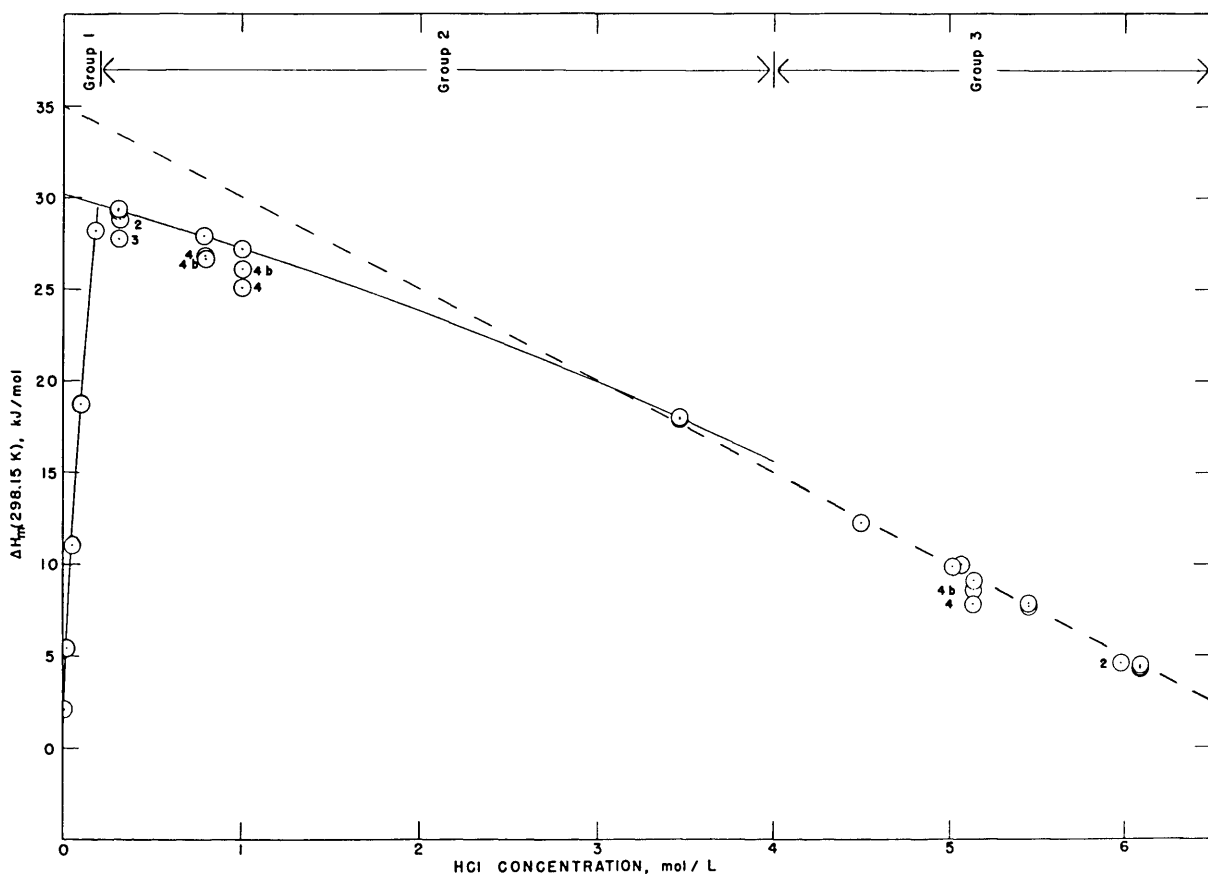


FIGURE 1. Plot of the values for the enthalpy of solution of guanine as a function of the concentration of the solvent, aqueous HCl. Group 1 shows the endothermic effect of dissolution of the guanine hydrochloride formed, Group 2 shows the exothermic effects of dilution, and the slope change in Group 3 probably indicates a protonation of the guanine molecule. Gua 1 was used for all experiments except those marked 2, 3, 4, and 4b which used other guanine samples for comparison. (see text).

Further justification for this grouping was provided by the differences in the electrical energy equivalents,  $\epsilon_i - \epsilon_f$ , listed in table 8 for the experiments in table 6 using Gua 1. The uncertainty in Group 1, where undissolved precipitate remained in the final solutions, was about one third of that in the other two groups. The large uncertainties in Groups 2 and 3 gives statistically questionable differences although the large difference between the means suggests a different reaction in the Group 3 experiments than in Group 2.

Table 8. Differences in energy equivalents for the initial and final systems ( $\epsilon_i - \epsilon_f$ ) for the measurements of the enthalpy of solution of Gua 1 in HCl (see table 6).

Group		
1	2	3
	J/K	
0.22	0.57	-0.20
.21	.76	-.12
.45	.00	.16
.20	.07	-.52
.25	.17	-.17
.41	.79	.28
		-.09
		-.16
		-.78
<sup>a</sup> 0.29 ± 0.04	<sup>a</sup> 0.39 ± 0.14	<sup>a</sup> - 0.18 ± 0.11

<sup>a</sup> Mean and standard deviation of the mean.

The values of  $\Delta H(298.15 \text{ K})$  as a function of HCl concentration for the experiments using Gua 1 (table 6) were fitted for each group by the method of least squares to linear, quadratic, and cubic equations. The resulting equations for the best fits are as follows ( $C$  is HCl concentration):

Group 1 ( $C = 0.01$  to  $0.2 \text{ mol} \cdot \text{L}^{-1}$ )

$$\Delta H(298.15 \text{ K}) = (0.04 \pm 0.43) + (229 \pm 13)(C) - (414 \pm 63)(C^2)$$

Standard deviation of the fit =  $0.42 \text{ kJ} \cdot \text{mol}^{-1}$ .

Group 2 ( $C = 0.3$  to  $3.5 \text{ mol} \cdot \text{L}^{-1}$ )

$$\Delta H(298.15 \text{ K}) = (30.181 \pm 0.032) - (2.705 \pm 0.060)(C) - (0.237 \pm 0.015)(C^2)$$

Standard deviation of the fit =  $0.025 \text{ kJ} \cdot \text{mol}^{-1}$ .

Combined Groups 2 and 3 ( $C = 0.3$  to  $6.1 \text{ mol} \cdot \text{L}^{-1}$ )

$$\Delta H(298.15 \text{ K}) = (30.28 \pm 0.19) - (2.85 \pm 0.16)(C) - (0.233 \pm 0.024)(C^2)$$

Standard deviation of the fit =  $0.27 \text{ kJ} \cdot \text{mol}^{-1}$ .

Group 3 ( $C = 4.5$  to  $6.1 \text{ mol} \cdot \text{L}^{-1}$ )

$$\Delta H(298.15 \text{ K}) = (34.98 \pm 0.75) - (5.00 \pm 0.14)(C)$$

Standard deviation of the fit =  $0.22 \text{ kJ} \cdot \text{mol}^{-1}$ .

The equation for the combined Groups 2 and 3 could be used, but it is believed that the use of separate equations is justified on the basis of the  $\Delta \epsilon_p$  and energy equivalent information discussed above. The two solid lines shown in figure 1 correspond to the equations for Groups 1 and 2, and the broken line, that for Group 3.

Beaven, et al [18], observed that adenine and guanine "have an additional pK in the region of pH 0." Our earlier work [5] reported evidence of such a protonation for adenine in HCl solution at the concentration,  $\sim 5 \text{ mol} \cdot \text{L}^{-1}$ , with  $\Delta H = (7.9 \pm 2.0) \text{ kJ} \cdot \text{mol}^{-1}$ . Apparently a similar protonation for guanine occurs at the HCl concentration, 3.5 to  $4 \text{ mol} \cdot \text{L}^{-1}$  (see fig. 1).  $\Delta H = (4.8 \pm 1.6) \text{ kJ} \cdot \text{mol}^{-1}$  was obtained from the difference between the intercepts of the equations for Group 3 and Group 2. The uncertainty is twice the sum of the standard deviations of the intercepts.

Several measurements of the enthalpy of solution using samples other than Gua 1 were made for comparison, and differences of about 10 percent (or more) in the values for  $\Delta H$  were observed. These results are also shown in figure 1. The values measured for Gua 2 were close to those of Gua 1, but those for Gua 3, 4, and 4b (the latter was reprecipitated from Gua 4), were significantly smaller than those of Gua 1. In two experiments, the values for Gua 4b were higher than comparable values for Gua 4. This suggests that impurities were removed in the reprecipitation of Gua 4b.

### 3.2. $\Delta H_{\text{soln}}$ of Gua Hydrochlorides in Aqueous HCl

Measurements of the enthalpy of solution in aqueous HCl at various concentrations were made on four samples of guanine hydrochloride described in section 2 and the subsections. The calorimetric measurements were made on the dried samples some of which were hygroscopic; therefore, transfers to the sample holder were all made in a dry atmosphere. The elemental analyses indicated that the dried Gua·HCl 1 and 2c were probably the anhydrous monohydrochloride, and that Gua·HCl 2d was closer to the composition of the anhydrous hemihydrochloride; there was no elemental analysis of Gua·HCl 2a, but it was assumed to be similar to 2c (which was heated at a somewhat higher temperature).

The data obtained in the experiments where the enthalpies of solution of the guanine hydrochlorides were measured are given in table 9; the arrangement is in order of increasing HCl concentration. The molar enthalpies of solution at the reference temperature given in the last column assume the sample composition indicated by the elemental analyses. These molar enthalpies are plotted in figure 2 as a function of the concentration of the aqueous HCl; also shown for comparison are the curves obtained for Group 2 and Group 3 in figure 1. In figure 2, the points representing the values for a given sample of Gua·HCl are connected by straight lines. The 3 points for Gua·HCl 2d (assuming it is

Table 9. Data from the measurements of the enthalpy of solution of guanine hydrochloride in aqueous HCl solutions.

Expt. No.	Gua·HCl		HCl Soln.			Reaction Period	Electrical Energy Equivalent		$-\Delta T_{\text{corr}}$	$-Q_{\text{obs}}$	$-q_v$	$-Q_r$	$T_r$	$^a\Delta H(298.15 \text{ K})$	
	No.	Mass	b No.	Conc.	Mass		$\epsilon_i$	$\epsilon_f$						J/g	kJ/mol
		g		mol/L	g	min	J/K		K	J	J	J	K		
1065	1	0.23895	51	0.307	302.43	115	1711.35	1710.13	0.021708	37.137	0.03	37.11	297.992	155.31	26.30
1059	2c	.12225	52	.312	302.45	10	1710.91	1710.82	.012104	20.709	.04	20.67	297.950	169.08	28.64
1057	2d	.20510	52	.312	302.46	25	1710.93	1710.32	.021145	36.172	.03	36.14	297.993	175.46	32.91 (29.72)
1064	1	.22370	(62)	.998	302.46	10	1658.62	1658.36	.020052	33.256	.03	33.26	298.019	148.68	25.18
1061	2c	.10652	(62)	2.83	307.50	30	1557.07	1556.11	.010003	15.570	.04	15.53	298.044	145.79	24.69
1060	1	.16306	(62)	2.84	307.64	20	1557.40	1557.23	.015960	24.856	.04	24.82	298.066	151.70	25.69
1062	1	.27484	(62)	4.00	307.61	55	1490.90	1490.40	.025175	37.527	.03	37.50	298.225	136.44	23.11
1063	1	.23858	(63)	4.99	307.45	25	1440.83	1440.62	.016145	23.260	.03	23.23	298.108	97.37	16.49
1056	1	.26366	60	5.01	307.40	20	1439.36	1439.60	.019347	27.850	.03	27.82	298.085	105.51	17.87
1055	2d	.18575	60	5.01	307.42	40	1439.05	1439.38	.011262	16.209	.03	16.18	298.111	87.10	16.34 (14.75)
1052	1	.21942	62	5.98	312.40	25	1410.82	1411.04	.016380	23.111	.03	23.08	298.102	105.19	17.82
1051	2a	.24960	61	5.98	312.42	135	1410.91	1411.02	.018812	26.543	.02	26.52	298.111	106.25	19.93
1053	2c	.10980	62	5.98	312.40	15	1410.46	1410.78	.008416	11.872	.04	11.83	298.101	107.74	18.25
1054	2d	.18211	62	5.98	312.43	35	1411.44	1411.13	.009121	12.872	.03	12.84	298.124	70.51	13.227 (11.941)

<sup>a</sup> The molar  $\Delta H$  is calculated on the assumption that the samples are the anhydrous hydrochloride (molar mass = 187.5883). The numbers in parentheses are based on the assumption that sample 2d is the hemihydrochloride (molar mass = 169.3578) as indicated by the elemental analysis.

<sup>b</sup> The HCl soln No. indicates the stock solution from which the calorimetric solution was taken. Numbers in parentheses indicate that the calorimetric solution was prepared by dilution of the stock solution of the given number.

<sup>c</sup> This includes a correction, 0.026 J, for a brief departure of the temperature of the adiabatic shield from that of the reaction vessel because of the rapid reaction.

the hemihydrochloride) are on a straight line and the slope is nearly the same as that for Group 2 in figure 1. The values obtained assuming the monohydrochloride composition are further away from the Group 2 curve of figure 1. The values for Gua·HCl 1 and 2c lie on a horizontal straight line up to the HCl concentration  $\sim 4 \cdot \text{mol} \cdot \text{L}^{-1}$ , but then there is a negative slope at the higher concentrations, somewhat analogous to that in figure 1. No conclusions can be drawn from these measurements because the molar quantities are based only on assumptions with respect to compositions. It does appear, however, that Gua·HCl 2d has different properties in solution than Gua·HCl 1 and 2c.

### 3.3. $\Delta H_{\text{soln}}$ of Gua in Aqueous NaOH

The results from 17 measurements of the enthalpy of solution of guanine in NaOH solutions are given in table 10 where the experiments are listed in order of increasing NaOH concentration. The sample masses are in the range 0.19 to 0.24 g (or 1.2 to 1.6 mmol of guanine), and the volume of the solvent is  $\sim 0.3 \text{ L}$ . The reactions were all rapid

and apparently complete; no residue was visible in the final solutions. In these experiments it was assumed that  $q_{\text{vap}}$  was negligibly small, because if the solutions were pure water  $q_{\text{vap}}$  would be  $< 0.05 \text{ J}$  in the worst case (at 309 K); the actual corrections would be smaller than this since the vapor pressure of  $\text{H}_2\text{O}$  over the NaOH solutions is less than that over pure  $\text{H}_2\text{O}$ .

Values for  $\Delta c_p$  of the reaction in NaOH solutions ( $\sim 0.1 \text{ mol} \cdot \text{L}^{-1}$ ) were obtained from Expts. No. 971 and 972,  $1.004 \text{ J} \cdot \text{g}^{-1} \cdot \text{K}^{-1}$ , and from Expts. No. 895 and 896,  $1.055 \text{ J} \cdot \text{g}^{-1} \cdot \text{K}^{-1}$ . For calculating  $\text{Corr}_{\text{sr}}$  the mean value,  $(1.03 \pm 0.05) \text{ J} \cdot \text{g}^{-1} \cdot \text{K}^{-1}$  was used; the uncertainty is estimated. The values may be different at the higher concentrations, but the work has terminated before other measurements could be made.

The values for the molar enthalpy of solution at the reference temperature,  $\Delta H_m(298.15 \text{ K})$ , from table 10 are plotted in figure 3 as a function of the concentration of the NaOH solution. Gua 1 was used for most of the measurements, but the numbers beside some of the points indicate the other guanine samples used (see sec. 2) for comparison. It can be

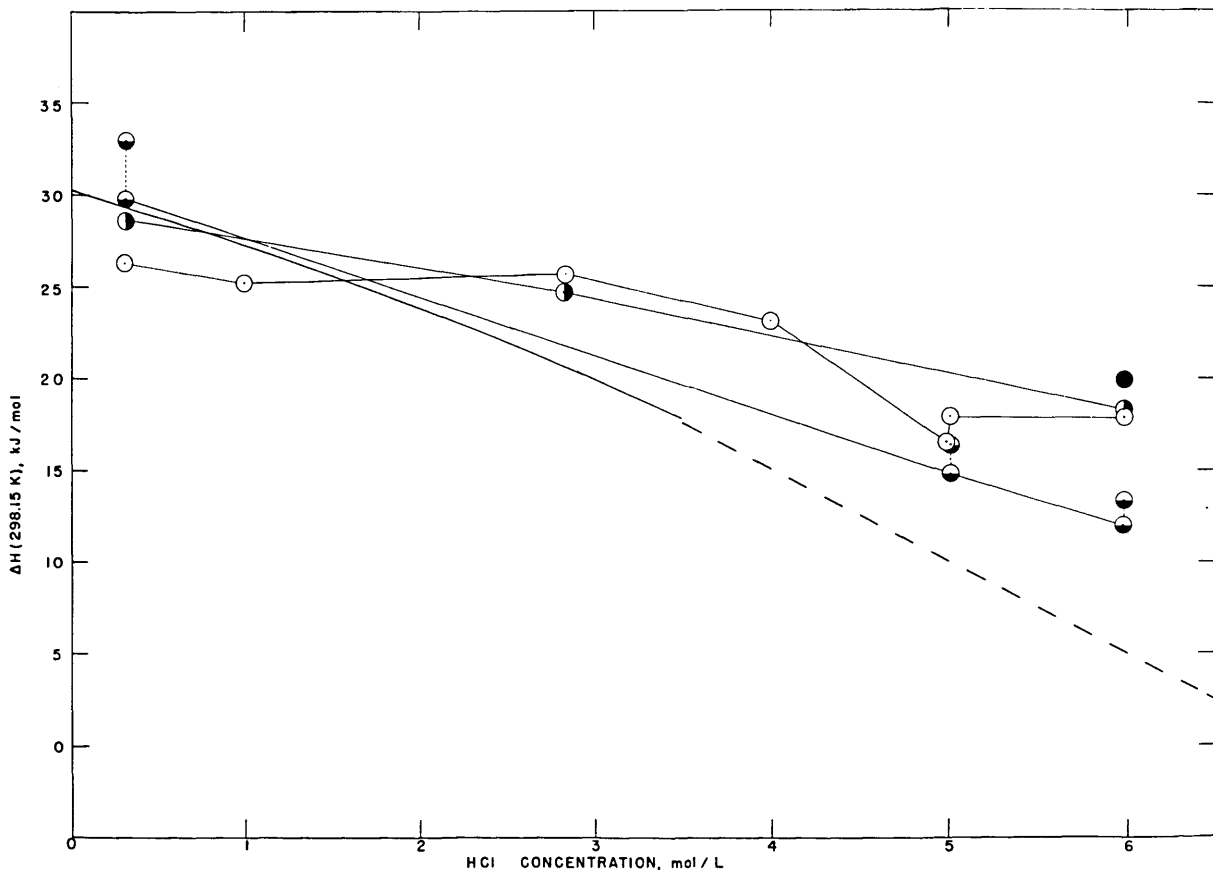


FIGURE 2. Plot of the values for the enthalpy of solution of various samples of guanine hydrochlorides as a function of the concentration of the solvent, aqueous HCl. The Gua·HCl samples are identified by the following symbols: O, 1; ●, 2a; ◐, 2c; and ●, 2d. The lower values of the points connected by dotted lines for Gua·HCl 2d were calculated assuming that the material was the hemihydrochloride (as indicated by the elemental analysis); the higher values were based on the assumption that the material was the monohydrochloride which appears to be an incorrect assumption. The curves from figure 1 are shown for comparison: Group 2, the solid line without data points, and Group 3, the broken line, without data points.

seen here that the values for Gua 2 were significantly less than those for Gua 1, but the values obtained in HCl solutions (see fig. 1) were nearly the same. Here the value for Gua 4 was close to those for Gua 1, but in the HCl solutions, Gua 4 showed a greater difference from Gua 1 than the other samples. Gua 4b, which was reprecipitated from Gua 4, had the lower values for the enthalpy of solution in aqueous NaOH; perhaps occluded water was not removed by drying.

A plot similar to figure 3 was previously given for adenine [5]. It showed no change of slope at  $\sim 0.5 \text{ mol} \cdot \text{L}^{-1}$  as shown in figure 3; the reason for this difference is not known. For adenine, there was a change of slope (more negative) at the NaOH concentration,  $\sim 4 \text{ mol} \cdot \text{L}^{-1}$ , which was assumed to be the result of a proton dissociation. Unfortunately, the guanine measurements terminated at  $\sim 3 \text{ mol} \cdot \text{L}^{-1}$ , although a proton dissociation similar to that for adenine would be expected. The following equation was obtained

from the fit of the data (table 10) for the 6 points above the NaOH concentration,  $C = 0.9 \text{ mol} \cdot \text{L}^{-1}$ , by the method of least squares to a linear equation:

$$\Delta H_m(298.15 \text{ K}) = (13.545 \pm 0.034) - (1.624 \pm 0.015)C.$$

The slope of this line is more negative than those from the adenine experiments,  $-0.36$  and  $-0.89 \text{ kJ} \cdot \text{mol}^{-1}$ . The standard deviation of the fit is  $0.036 \text{ kJ} \cdot \text{mol}^{-1}$ .

#### 4. Discussion and Summary

Few measurements of the thermodynamic properties of guanine have been made. Stiehler and Huffman measured the heat of combustion [7], and the heat capacity [19] from which they calculated the entropy and free energy. The enthalpy of sublimation was measured by Yanson and Teplit-

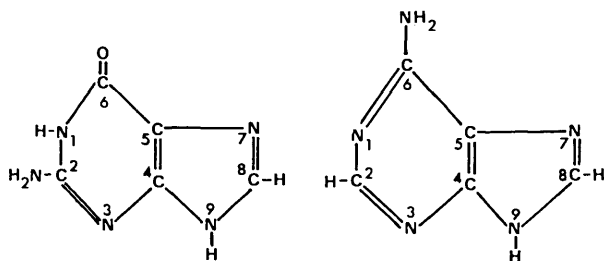
Table 10. Data from the measurements of the enthalpy of solution of guanine in aqueous NaOH solutions.

Expt. No.	Sample	Gua <sup>a</sup> Mass g	Soln. No.	NaOH		Reaction Period min	Electrical Energy Equivalent		$-\Delta T_{\text{obs}}$ K	$-Q_{\text{obs}}$ J	$-Q_r$ J/g	$T_r$ K	Corr. <sub>ST</sub> J/g	$\Delta H_m(298.15 \text{ K})$	
				Conc. mol/L	Mass g		$\epsilon_i$	$\epsilon_f$						J/g	kJ/mol
971	1	0.20668	44	0.0912	297.41	12	1709.88	1708.82	0.013536	23.138	111.95	298.226	0.08	111.87	16.91
972	1	.23756	44	.0912	302.40	12	1730.02	1730.00	.016860	29.167	122.78	309.009	11.18	111.60	16.86
895	1	.20809	35	.1010	303.62	7	1735.30	1734.67	.012994	22.545	108.34	298.208	0.06	108.28	16.36
896	1	.19833	35	.1010	303.64	5	1735.48	1735.21	.013658	23.702	119.51	308.795	10.96	108.55	16.40
973	1	.20837	42	.462	302.33	17	1704.99	1704.81	.010618	18.103	86.88	298.127	-0.02	86.90	13.13
894	1	.21029	36	.514	303.65	8	1707.49	1707.61	.01048	17.888	85.06	298.128	-.02	85.08	12.86
974	1	.21592	41	.919	302.20	12	1679.89	1679.70	.010263	17.240	79.84	298.140	-.01	79.85	12.07
1046	1	.20353	46	1.02	308.56	12	1699.42	1699.18	.009418	16.005	78.64	298.150	-.01	78.65	11.89
1048	4b	.20134	46	1.02	308.47	10	1698.46	1698.34	.009054	15.377	76.37	298.135	-.02	76.39	11.54
1045	1	.19545	45	1.33	308.56	7	1686.00	1685.90	.008716	14.694	75.18	298.146	.00	75.18	11.36
1047	4b	.19867	45	1.33	308.46	12	1686.01	1685.35	.008609	14.512	73.05	298.146	.00	73.05	11.04
1049	4	.19847	45	1.33	308.44	10	1685.36	1685.40	.008939	15.066	75.91	298.136	-.02	75.93	11.48
1050	2	.20435	45	1.33	308.47	10	1684.96	1685.26	.007805	13.153	64.37	298.240	.09	64.28	9.71
1050A	2	.19805	45	1.33	308.45	10	1685.62	1685.41	.007587	12.788	64.57	298.146	.00	64.57	9.76
975	1	.22021	43	2.83	307.15	12	1634.91	1634.75	.007988	13.059	59.30	298.153	.00	59.30	8.96
892	1	.19156	39	3.12	313.67	22	1654.22	1654.6	<sup>b</sup> -.511124	10.787	56.31	298.095	-.06	56.37	8.52
893	1	.19357	39	3.12	313.48	7	1653.23	1653.44	.006525	10.788	55.73	298.094	-.06	55.79	8.43

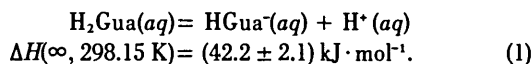
<sup>a</sup> Corrected for H<sub>2</sub>O determined by vacuum drying other portions of the sample as follows: (Dried at 370 K) Gua 1, 1.01 % and Gua 4, 1.70 % (Dried at 340 K), Gua 2, 1.5 %. The Gua 4b samples had been dried at 340 K and no corrections were made for H<sub>2</sub>O in the samples.

<sup>b</sup> Electrical energy added during the reaction = 856.405 J.

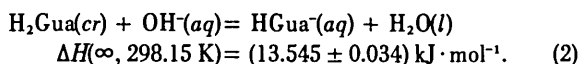
skii [20]. Measurements of pK values for protonation and proton dissociation by electrometric titration and by spectrophotometry were summarized by Izatt, et al. [21]. This review also discusses the protonation sites, N7 being the most likely, and the proton dissociation site, N1, in the guanine molecule. The structures of two of the purine bases, guanine and adenine, were given as



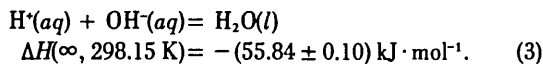
No values were reported for the enthalpy of protonation and only one value for the enthalpy of proton dissociation obtained from spectrophotometric measurements over large pH and temperature ranges. The value obtained by Suchorukow, et al. [22], may be described with the following equation which is analogous to those given for adenine [1]:



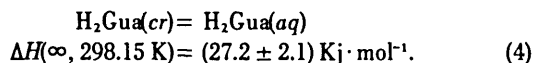
Our measurements reported in section 3.3 result in



Parker's selected value for  $\Delta H_r^\circ$  [17] can be represented by



If the sum of eq (1) and (3) is subtracted from eq (2), we obtain the enthalpy of solution of crystalline guanine at infinite dilution,



Here and subsequently, the calculated uncertainty is the square root of the sum of the squares of the individual uncertainties.

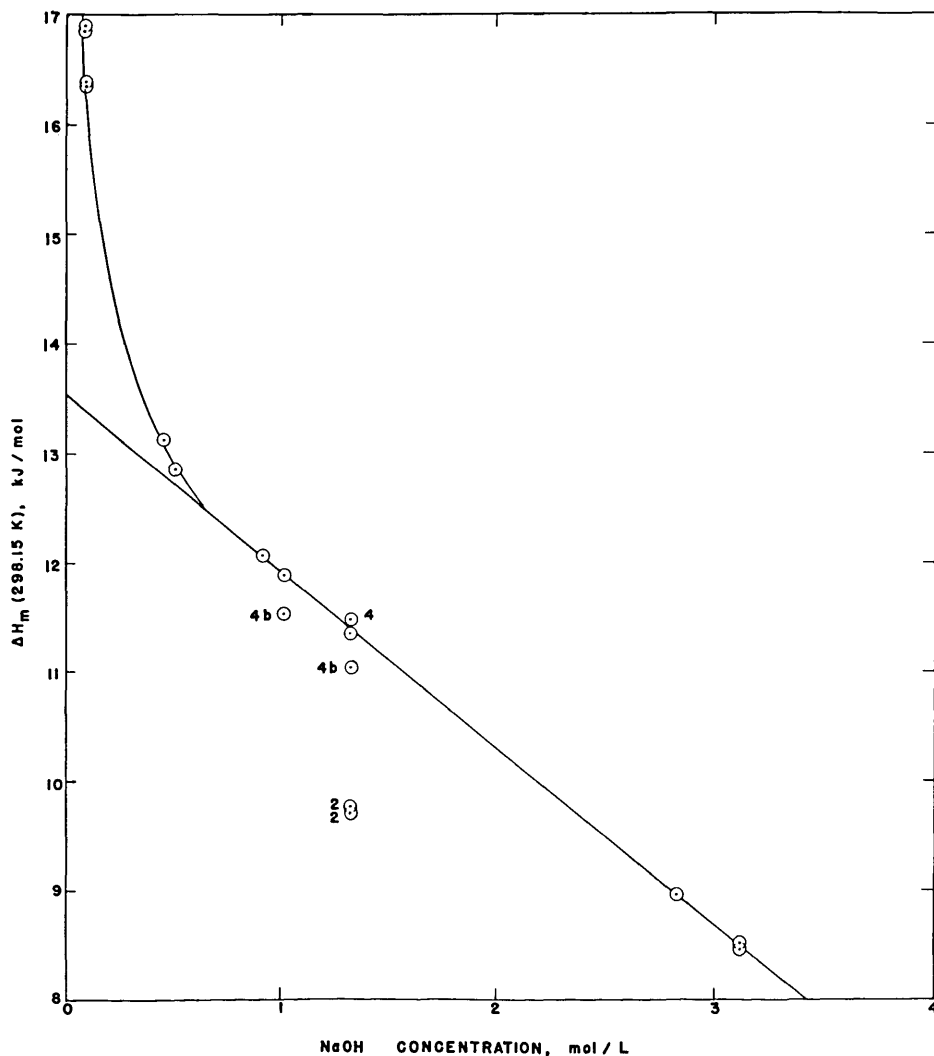


FIGURE 3. Plot of the enthalpy of solution of guanine as a function of the concentration of the solvent, aqueous NaOH. Gua 1 was used in all measurements except those with a number indicating the guanine sample used (see sec. 2).

Miller and Wasik [23] measured the solubility of our crystalline guanine sample, Gua 1, at three temperatures using a liquid chromatographic method developed by Wasik which is described elsewhere [24]. The guanine was first dissolved in methanol as a carrier liquid and transferred to the chromatographic column. The methanol was removed under vacuum, liquid water was introduced for saturation by the guanine deposit, and the guanine concentration in the saturated solution was determined chromatographically. The results are as follows: mean of 3 measurements at 292 K,  $(5.0 \pm 0.2) \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ ; mean of 8 measurements at 298 K,  $(6.0 \pm 0.2) \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ ; and mean of 3 measurements at 303 K,  $(8.7 \pm 0.3) \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ . The uncertainties are the average deviations. The enthalpy of solution,

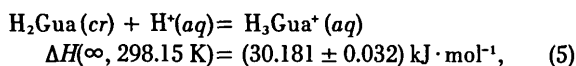
$\Delta H$ , may be calculated from the solubilities using the van't Hoff equation,

$$\frac{d \ln s}{dT} = \frac{\Delta H}{RT^2},$$

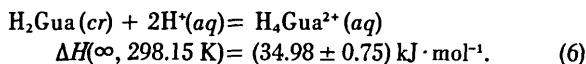
where  $s$  is the solubility,  $T$  is the absolute temperature, and  $R$  is the gas constant. Assuming the  $\Delta H$  is linear with temperature we calculate a value of  $\sim 36 \text{ kJ} \cdot \text{mol}^{-1}$  at 298 K. This is in disappointingly poor agreement with our value at infinite dilution calculated in equation (4), which would probably be nearly the same as the value at saturation because the solubility is extremely low. It is possible that

some chemical change in the guanine occurred when it was dissolved in methanol, and that the measurements were actually made on a derivative of guanine. Therefore, a second set of measurements were made in which the presolution in methanol was by-passed. A saturated aqueous solution was introduced directly into the column and its concentration was determined. Three measurements were made at each of three temperatures and the average solubilities are as follows:  $3.5 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$  at 293 K,  $4.5 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$  at 298 K, and  $4.8 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$  at 303 K. The average deviations were less than  $1 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$ .  $\Delta H = 22 \text{ kJ} \cdot \text{mol}^{-1}$  was calculated by the above method. This is *lower* than our value in eq (4), possibly because of the difficulty in saturating the solution. It is noteworthy that the solubilities in water were different if the guanine was pretreated in methanol.

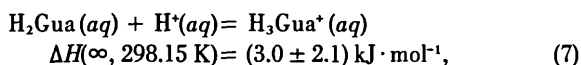
In reference [21] evidence was given for the protonation at N7. Our measurements in section 3.1 show, in addition, a second protonation (probably at N3) at  $\text{pH} < 1$ . These correspond to the following equations:



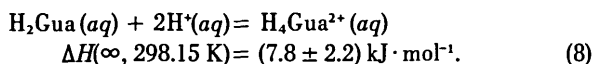
and



By subtracting eq(4) from eq(5) and from eq(6), we obtain



and



Equation (7) corresponds to the protonation of N7 and eq (8) presumably to the protonation of both N7 and N3. No other values have been reported previously for these reactions.

Analogous enthalpy of solution values obtained for adenine in [1] and for guanine in this work are summarized in table 11. A summary of values measured for  $\Delta C_p$  of reaction for adenine and guanine are given in table 12.

The results reported here have shown the need for thorough characterization of the samples used in precise calorimetric measurements. More work is needed before reliable values for the guanine hydrochlorides can be given.

Table 11. Summary of analogous values for enthalpies of solution, protonation, and proton dissociation for the purine bases, adenine and guanine.

Equation (P = purine base, Ade or Gua)	$\Delta H(\infty, 298.15 \text{ K})$	
	Ade [1]	Gua [This work]
	kJ/mol	
$\text{H}_2\text{P}(\text{cr}) + \text{aq} = \text{H}_2\text{P}(\text{aq})$	33.47	<sup>a</sup> 2.72
$\text{H}_2\text{P}(\text{cr}) + \text{H}^+(\text{aq}) = \text{H}_3\text{P}^+(\text{aq})$	11.41	30.181
$\text{H}_2\text{P}(\text{cr}) + 2 \text{H}^+(\text{aq}) = \text{H}_4\text{P}^{2+}(\text{aq})$	19.3	34.98
$\text{H}_2\text{P}(\text{cr}) + \text{OH}^-(\text{aq}) = \text{HP}^-(\text{aq}) + \text{H}_2\text{O}(\ell)$	15.207	13.545
$\text{H}_2\text{P}(\text{cr}) + 2 \text{OH}^-(\text{aq}) = \text{P}^{2-}(\text{aq}) + 2 \text{H}_2\text{O}(\ell)$	17.26	---
$\text{H}_2\text{P}(\text{aq}) + \text{H}^+(\text{aq}) = \text{H}_3\text{P}^+(\text{aq})$	-22.1	<sup>a</sup> 3.0
$\text{H}_2\text{P}(\text{aq}) + 2 \text{H}^+(\text{aq}) = \text{H}_4\text{P}^{2+}(\text{aq})$	-14.2	<sup>a</sup> 7.8
$\text{H}_2\text{P}(\text{aq}) = \text{HP}^-(\text{aq}) + \text{H}^+$	<sup>a</sup> 37.58	<sup>b</sup> (42.2)
$\text{H}_2\text{P}(\text{aq}) = \text{P}^{2-}(\text{aq}) + 2 \text{H}^+$	<sup>a</sup> 95.47	---

<sup>a</sup> Calculated using auxiliary data (other values obtained by direct measurements).

<sup>b</sup> Obtained by Suchorukow, et al. [22].

Table 12. Summary of values of the  $\Delta C_p$  of reaction measured for adenine and guanine in various solvents at 298 K. (Uncertainties are estimated except as noted).

Solvent	Ade [1,5]		Gua [this work]	
	Solvent Conc. mol/L	$\Delta C_p$ J/mol·K	Solvent Conc. mol/L	$\Delta C_p$ J/mol·K
HCl			0.1	$814 \pm 75$
	1.0	$107 \pm 3$	0.3 to 3.5	$98 \pm 8$
			$\sim 6$	$160 \pm 15$
NaOH	0.9	$126 \pm 8$	0.1	$156 \pm 8$
H <sub>2</sub> O	---	<sup>a</sup> $78.7 \pm 10.4$	---	---

<sup>a</sup> Uncertainty is at the 95 % confidence level.

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